
AMENDMENTS TO THE SPECFICATION:

Pursuant to 37 C.F.R. § 1.121, please amend the specification as follows. Additions are indicated by underlining and deletions are indicated by **~~bold strikethrough~~.**

Please amend the specification to insert the Sequence Listing filed on June 27, 2003 at the end of the specification.

Please replace the paragraph beginning at page 1, line 4 with the following amended paragraph:

This application is a Continuation of US Application Serial No. 10/084,706 filed February 26, 2002, now U.S. Patent No. 7,144,574, which claims priority from and benefit of U.S. Provisional Application No. 60/272,116 filed February 27, 2001, U.S. Provisional Application No. 60/343,436 filed December 21, 2001, U.S. Provisional Application No. 60/302,140 filed June 29, 2001, U.S. Provisional Application No. 60/316,170 filed August 30, 2001, and U.S. Provisional Application No. 60/357,945 filed February 19, 2002. Pursuant to 35 U.S.C. §119(a)-(d), USSN 10/084,706 also claims priority from and benefit of Danish Patent Application No. PA 2001 00333 filed March 1, 2001. The disclosure of each application listed above is incorporated herein in its entirety for all purposes.

Please replace the paragraph beginning at page 2, line 27 with the following amended paragraph:

Relatively few protein-engineered variants of IFNB have been reported (WO 9525170, WO 9848018, US 5545723, US 4914033, EP 260350, US 4588585, US 4769233, Stewart et al., DNA, Vol. 6, No. 2, 1987, ~~**al, DNA Vol 6 no2 1987**~~ pp. 119-128, Runkel et al., ~~**al,**~~ 1998, J. ~~**Jour.**~~ Biol. Chem. 273, No. 14, pp. 8003-8008).

Please replace the paragraph beginning at page 3, line 1 with the following amended paragraph:

Redlich et al., ~~al,~~ Proc. Natl. Acad. Sci., USA, Vol. 88, pp. 4040-4044, 1991 disclose immunoreactivity of antibodies against synthetic peptides corresponding to peptide stretches of recombinant human IFNB with the mutation C17S.

Please replace the paragraph beginning at page 3, line 27 with the following amended paragraph:

Commercial preparations of IFNB are sold under the names Betaseron® interferon beta-1b (also termed interferon β1b, which is non-glycosylated, produced using recombinant bacterial cells, has a deletion of the N-terminal methionine residue and the C17S mutation), and Avonex® interferon beta-1a ~~Avonex™~~ and Rebif® interferon beta-1a (also termed interferon β1a, which is glycosylated, produced using recombinant mammalian cells) for treatment of patients with multiple sclerosis, have shown to be effective in reducing the exacerbation rate, and more patients remain exacerbation-free for prolonged periods of time as compared with placebo-treated patients. Furthermore, the accumulation rate of disability is reduced (Neurol. 51:682-689, 1998).

Please replace the paragraph beginning at page 12, line 24 with the following amended paragraph:

In the present application, amino acid names and atom names (e.g. CA, CB, CD, CG, SG, NZ, N, O, C, etc) are used as defined by the Protein DataBank (PDB) (www website at pdb.org) ~~(www.pdb.org)~~ which are based on the IUPAC nomenclature (IUPAC Nomenclature and Symbolism for Amino Acids and Peptides (residue names, atom names, etc. ~~e.t.c.~~), *Eur. J. Biochem.*, 138, 9-37 (1984) together with their corrections in *Eur. J. Biochem.*, 152, 1 (1985). CA is sometimes referred to as Cα, CB as Cβ. The term "amino acid residue" is intended to indicate an amino acid residue contained in the group consisting of alanine (Ala or A), cysteine (Cys or C), aspartic acid (Asp or D), glutamic acid (Glu or E), phenylalanine (Phe or F), glycine (Gly or G), histidine (His or H), isoleucine (Ile or I), lysine (Lys or K), leucine (Leu or L), methionine (Met or M), asparagine (Asn or N), proline (Pro or P), glutamine (Gln or Q), arginine (Arg or R), serine (Ser or S), threonine (Thr or T), valine (Val or V), tryptophan (Trp or W), and

tyrosine (Tyr or Y) residues. The terminology used for identifying amino acid positions/substitutions is illustrated as follows: C17 (indicates position #17 occupied by a cysteine residue in the amino acid sequence shown in SEQ ID NO:2). C17S (indicates that the cysteine residue of position 17 has been replaced with a serine). The numbering of amino acid residues made herein is made relative to the amino acid sequence shown in SEQ ID NO:2. "M1del" is used about a deletion of the methionine residue occupying position 1. Multiple substitutions are indicated with a "+", e.g. R71N+D73T/S means an amino acid sequence which comprises a substitution of the arginine residue in position 71 with an asparagine and a substitution of the aspartic acid residue in position 73 with a threonine or serine residue, preferably a threonine residue. T/S as used about a given substitution herein means either a T or S residue, preferably a T residue.

Please replace the paragraph beginning at page 14, line 21 with the following amended paragraph:

The term "immunogenicity" as used in connection with a given substance is intended to indicate the ability of the substance to induce a response from the immune system. The immune response may be a cell or antibody mediated response (see, e.g., Roitt: Essential Immunology (8^{th} Edition, Blackwell) for further definition of immunogenicity). Immunogenicity may be determined by use of any suitable method known in the art, e.g. *in vivo* or *in vitro,* e.g. using the *in vitro* immunogenicity test outlined in the Materials and Methods section below. The term "reduced immunogenicity" as used about a given polypeptide or conjugate is intended to indicate that the conjugate or polypeptide gives rise to a measurably lower immune response than a reference molecule, such as wildtype human IFNB, e.g., Rebif® interferon beta-1a or Avonex® interferon beta-1a, or a variant of wild-type human IFNB such as Betaseron® interferon beta-1b, as determined under comparable conditions. When reference is made herein to commercially available IFNB products (i.e. Betaseron® interferon beta-1b, Avonex® interferon beta-1a and Rebif® interferon beta-1a), it should be understood to mean either the formulated product or the IFNB polypeptide part of the product (as appropriate). Normally, reduced antibody reactivity

(e.g. reactivity towards antibodies present in serum from patients treated with commercial IFNB products) is an indication of reduced immunogenicity.

Please replace the paragraph beginning at page 16, line 3 with the following amended paragraph:

The term "increased" as used about the functional *in vivo* half-life or serum half-life is used to indicate that the relevant half-life of the conjugate or polypeptide is statistically significantly increased relative to that of a reference molecule, such as an un-conjugated wildtype human IFNB (e.g., Avonex® interferon beta-1a or Rebif® interferon beta-1a) or an unconjugated variant human IFNB (e.g., Betaseron® interferon beta-1b) as determined under comparable conditions.

Please replace the paragraph beginning at page 17, line 9 with the following amended paragraph:

The term "parent IFNB" is intended to indicate the starting molecule to be improved in accordance with the present invention and/or as described in co-pending U.S. Application Serial No. (U.S.S.N.) 09/648,569. Preferably, the parent IFNB belongs to the IFNB sequence family. While the parent IFNB may be of any origin, such as vertebrate or mammalian or primate origin (e.g. any of the origins defined in WO 00/23472), the parent IFNB is preferably wild-type human IFNB with the amino acid sequence shown in SEQ ID NO:2 or a variant thereof. In the context of a parent IFNB polypeptide, a "variant" is a polypeptide, which differs in one or more amino acid residues from a parent polypeptide, normally in 1, 2, 3, ~~2,3,~~ 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acid residues. Examples of wild-type human IFNB include the polypeptide part of Avonex® interferon beta-1a or Rebif® interferon beta-1a. An example of a parent IFNB variant is Betaseron® interferon beta-1b. Alternatively, the parent IFNB polypeptide may comprise an amino acid sequence, which is a hybrid molecule between IFNB and another homologous polypeptide, such as interferon α, interferon τ, or interferon ω, optionally containing one or more additional substitutions introduced into the hybrid molecule. Such a hybrid molecule may contain an amino acid sequence, which differs in more than 10 amino acid residues from the amino acid sequence shown in SEQ ID NO:2. In order to be useful as a parent polypeptide the

hybrid molecule exhibits IFNB activity (e.g. as determined in the secondary assay described in the Materials and Methods section herein). Other examples of variants of wild-type human IFNB that may serve as parent IFNB molecules in the present invention include, for example: polypeptides described in U.S.S.N. 09/648,569 having introduced and/or removed amino acid residues comprising an attachment group for a non-polypeptide moiety, and any IFNB molecule described in WO 00/23114, WO 00/23472, WO 99/3887. Any wild-type IFNB or variant thereof available in the art may serve as a parent IFNB molecule in the present invention.

Please replace the paragraph beginning at page 21, line 21 with the following amended paragraph:

In yet another embodiment the variant is prepared from a parent IFNB polypeptide comprising an introduced glycosylation site defined by a substitution equivalent to or being S2N+N4T/S of SEQ ID NO:2, the variant further comprising a substitution of the amino acid residue located in an equivalent position to or being M1, Y3 or L5 of SEQ ID NO:2, the substitution being made to an amino acid residue which gives rise to increased glycosylation at said introduced glycosylation site as compared to that of the parent IFNB polypeptide. Preferably, the amino acid residue to be substituted is located in a position equivalent to or being M1. By use of the www website at cbs.dtu.dk/services/SignalP/, ~~http://www.cbs.dtu.dk/services/SignalP/)~~ it has been verified that all amino acid substitutions are allowed in position 1 of SEQ ID NO:2 (i.e. allows for correct signal peptide cleavage).

Please replace the paragraph beginning at page 27, line 3 with the following amended paragraph:

In a further embodiment the glycosylated interferon β polypeptide comprises one to five sugar moieties, such as one to three sugar moieties. When the interferon molecule is glycosylated it is preferably N-glycosylated. When the interferon molecule is glycosylated it usually comprises 1-5 sugar moieties, such as 1-3 sugar moieties. In a further embodiment, the interferon molecule is N-glycosylated, and comprises 1-5 sugar moieties, such as 1-3 sugar moieties. In a further embodiment, the interferon molecule is N-glycosylated, and comprises 3 sugar moieties.

According to the specific aspects above, the interferon β polypeptide has three sugar moieties, that are ~~is~~ in positions ~~position~~ N49, N80, and N111.

Please replace the paragraph beginning at page 34, line 1 with the following amended paragraph:

Reduced immunogenicity as compared to wild-type human IFNB (e.g. Avonex® interferon beta-1a or Rebif® interferon beta-1a) or to Betaseron® interferon beta-1b, e.g. a reduction of at least 25%, such as at least 50%, and more preferably at least 75%;

Please replace the paragraph beginning at page 34, line 4 with the following amended paragraph:

Increased functional *in vivo* half-life and/or increased serum half-life as compared to wild-type human IFNB (e.g. Avonex® interferon beta-1a or Rebif® interferon beta-1a) or to Betaseron® interferon beta-1b;

Please replace the paragraph beginning at page 34, line 6 with the following amended paragraph:

Reduced or no reaction with neutralizing antibodies from patients treated with wildtype human IFNB (e.g. Rebif® interferon beta-1a or Avonex® interferon beta-1a) or with Betaseron® interferon beta-1b, e.g. a reduction of neutralisation of at least 25%, such as of at least 50%, and preferably of at least 75% as compared to the wildtype human IFNB.

Please replace the paragraph beginning at page 34, line 10 with the following amended paragraph:

The magnitude of the antiviral activity of a conjugate of the invention may not be critical, and thus be reduced (e.g. by up to 75%) or increased (e.g. by at least 5%) or equal to that of wild-type human IFNB ((e.g. Avonex® interferon beta-1a or Rebif® interferon beta-1a) or to Betaseron® interferon beta-1b as determined under comparable conditions.

Please replace the paragraph beginning at page 49, line 19 with the following amended

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paragraph:

As indicated further above the non-polypeptide moiety of the conjugate of the invention is preferably selected from the group consisting of a polymer molecule, a lipophilic compound, a sugar moiety (by way of *in vivo* glycosylation) and an organic derivatizing agent. All of these agents may confer desirable properties to the polypeptide part of the conjugate, in particular reduced immunogenicity and/or increased functional *in vivo* half-life and/or increased serum half-life. The polypeptide part of the conjugate may be conjugated to only one type of non-polypeptide moiety, but may also be conjugated to two or more different types of non-polypeptide moieties, e.g. to a polymer molecule and a sugar moiety, to a lipophilic group and a sugar moiety, to an organic derivatizing ~~derivating~~ agent and a sugar moiety, to a lipophilic group and a polymer molecule, etc. The conjugation to two or more different non-polypeptide moieties may be done simultaneous or sequentially. The choice of non-polypeptide moiety/ies, e.g. depends on the effect desired to be achieved by the conjugation. For instance, sugar moieties have been found particularly useful for reducing immunogenicity, whereas polymer molecules such as PEG are of particular use for increasing functional *in vivo* half-life and/or serum half-life. Using a polymer molecule as a first non-polypeptide moiety and a sugar moiety as a second non-polypeptide moiety ~~moiey~~ may result in reduced immunogenicity and increased functional *in vivo* or serum half-life.

Please replace the paragraph beginning at page 50, line 12 with the following amended paragraph:

For conjugation to a lipophilic compound the following polypeptide groups may function as attachment groups: the N-terminal or C-terminal of the polypeptide, the hydroxy groups of the amino acid residues Ser, Thr or Tyr, the ε-amino group of Lys, the SH group of Cys or the carboxyl group of Asp and Glu. The polypeptide and the lipophilic compound may be conjugated to each other, either directly or by use of a linker. The lipophilic compound may be a natural compound such as a saturated or unsaturated fatty acid, a fatty acid diketone, a terpene, a prostaglandin, a vitamin ~~vitamine~~, a carotenoid ~~carotenoide~~ or steroid ~~steroide~~, or a synthetic compound such as a carbon acid, an alcohol, an amine and sulphonic acid with one or more

alkyl-, aryl-, alkenyl- or other multiple unsaturated compounds. The conjugation between the polypeptide and the lipophilic compound, optionally through a linker may be done according to methods known in the art, e.g. as described by Bodanszky in Peptide Synthesis, John Wiley, New York, 1976 and in WO 96/12505.

Please replace the paragraph beginning at page 54, line 10 with the following amended paragraph:

Covalent *in vitro* coupling of a carbohydrate moiety to amino acid residues of IFNB may be used to modify or increase the number or profile of carbohydrate substituents. Depending on the coupling mode used, the carbohydrate(s) may be attached to a) arginine and histidine (Lundblad and Noyes, Chemical Reagents for Protein Modification, CRC Press Inc. Boca Raton, FI), b) free carboxyl groups (e.g. of the C-terminal amino acid residue, asparagine or glutamine), c) free sulfhydryl groups such as that of cysteine, d) free hydroxyl groups such as those of serine, threonine, tyrosine or hydroxyproline, e) aromatic residues such as those of phenylalanine or tryptophan or f) the amide group of glutamine. These amino acid residues constitute examples of attachment groups for a carbohydrate moiety, which may be introduced and/or removed in the IFNB polypeptide. Suitable methods of *in vitro* coupling are described in WO 87/05330 and in Aplin et ~~etl~~ al., CRC Crit. ~~Crit~~ Rev. Biochem., pp. 259-306, 1981. The *in vitro* coupling of carbohydrate moieties or PEG to protein- and peptide-bound Gln-residues can also be carried out by transglutaminases (TGases), e.g. as described by Sato et al., 1996 Biochemistry 35, 13072-13080 or in EP 725145.

Please replace the paragraph beginning at page 54, line 28 with the following amended paragraph:

In order to achieve *in vivo* glycosylation of an IFNB polypeptide as described herein, e.g. one that has been modified by introduction of one or more glycosylation sites (see the section "Conjugates of the invention wherein the non-polypeptide moiety is a sugar moiety") or by modification of an amino acid residue located close to a glycosylation site (as described in the section entitled "Variants with increased glycosylation"), the nucleotide sequence encoding the polypeptide part of the conjugate must be inserted in a glycosylating, eukaryotic ~~eucaryotic~~

expression host. The expression host cell may be selected from fungal (filamentous fungal or yeast), insect, mammalian, animal and transgenic plant cells or from transgenic animals. Furthermore, the glycosylation may be achieved in the human body when using a nucleotide sequence encoding a polypeptide described herein in gene therapy. In one embodiment the host cell is a mammalian cell, such as an CHO cell, BHK or HEK cell, e.g. HEK293, or an insect cell, such as an SF9 cell, or a yeast cell, e.g. *Saccharomyces cerevisiae, Pichia pastoris* or any other suitable glycosylating host, e.g. as described further below. Optionally, sugar moieties attached to the IFNB polypeptide by *in vivo* glycosylation are further modified by use of glycosyltransferases, e.g. using the glycoAdvance™ technology marketed by Neose, Horsham, PA, USA. Thereby, it is possible to, e.g., increase the sialylation ~~sialyation~~ of the glycosylated IFNB polypeptide following expression and *in vivo* glycosylation by CHO cells.

Please replace the paragraph beginning at page 55, line 16 with the following amended paragraph:

Covalent modification of the IFNB polypeptide may be performed by reacting (an) attachment group(s) of the polypeptide with an organic derivatizing agent. Suitable derivatizing agents and methods are well known in the art. For example, cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(4-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole. Histidyl residues are derivatized by reaction with diethylpyrocarbonate at ~~diethylpyrocarbonateat~~ pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0.Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing α-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal;

chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate. Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine guanidino group. Carboxyl side groups (aspartyl or glutamyl or C-terminal amino acid residue) are selectively modified by reaction with carbodiimides (R-N=C=N-R'), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Please replace the paragraph beginning at page 58, line 13 with the following amended paragraph:

In an alternative embodiment the interferon β polypeptide is expressed, as a fusion protein, with a tag, i.e. an amino acid sequence or peptide stretch made up of typically 1-30, such as 1-20 or 1-15 or 1-10 amino acid residues. Besides allowing for fast and easy purification, the tag is a convenient tool for achieving conjugation between the tagged <u>polypeptide and</u> ~~**polypeptideand**~~ the non-polypeptide moiety. In particular, the tag may be used for achieving conjugation in microtiter plates or other carriers, such as paramagnetic beads, to which the tagged polypeptide can be immobilised via the tag. The conjugation to the tagged polypeptide in, e.g., microtiter plates has the advantage that the tagged polypeptide can be immobilised in the microtiter plates directly from the culture broth (in principle without any purification) and subjected to conjugation. Thereby, the total number of process steps (from expression to conjugation) can be reduced. Furthermore, the tag may function as a spacer molecule ensuring an improved accessibility to the immobilised polypeptide to be conjugated. The conjugation using a tagged polypeptide may be to any of the non-polypeptide moieties disclosed herein, e.g. to a polymer molecule such as PEG.

Please replace the paragraph beginning at page 60, line 10 with the following amended paragraph:

In a further aspect the invention relates to an interferon β polypeptide comprising an amino acid sequence which differs from that of wild-type human interferon P in that at least one amino acid residue selected from the group consisting of N4, F8, L9, Q10, R11, S12, S13, L24, N25, G26, L28, E29, N37, F38, D39, Q48, Q49, Q64, N65, I66, F67, A68, i69, F70, R71, Q72, D73, S74, S75, S76, T77, G78, W79, N80, E81, T82,183, V84, E85, L87, L88, A89, N90, V91, Y92, H93, Q94, D110, F111, T112, R113, R128, H140, T144, 1145, R147, V148, L151, R152, F154, Y155, N158, G162, and N166 is replaced with a lysine residue, provided that the polypeptide is different from the one having the amino acid sequence of wild-type human interferon β with the following substitutions: D54N+E85K+V91I+V101M and different from one which is a hybrid molecule between interferon β and interferon α which as a consequence of being a hybrid has a lysine in position 39. The first of the disclaimed polypeptides is disclosed by Stewart et al., DNA, Vol. 6, No. 2, 1987, pp. 119-128 ~~al, DNA Vol 6 no2 1987 p 119-128~~ and was found to be inactive, the second is disclosed in US 4,769,233 and was constructed with the purpose of improving the biological activity of interferon β. None of the disclaimed polypeptides were made for or described as being suitable intermediates for the preparation of interferon β conjugates with reduced immunogenicity and/or prolonged functional in vivo half-life and/or serum half-life.

Please replace the paragraph beginning at page 60, line 28 with the following amended paragraph:

A still further example includes an interferon β polypeptide comprising an amino acid sequence which differs from that of SEQ ID NO:2 in one or more substitutions selected from the group consisting of N4K, F15K, Q16K, R27K, R35K, D39K, Q49K, E85K, A89K, E103K, E109K, R124K, E137K and R159K, provided that when the substitution is R27K the polypeptide is different from the one having the amino acid sequence of wild-type human interferon β with the following substitutions: R27K+E43K. The disclaimed polypeptide is disclosed by Stewart et al., DNA. Vol. 6, No. 2, 1987, pp. 119-128 ~~al, DNA Vol 6 no2 1987 p119-128~~ and was found to

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have a low activity. The polypeptide was made in the course of a study of function- structure relationship and was not mentioned as a possible intermediate product for the preparation of improved interferon β conjugate molecules. For instance, the interferon β polypeptide comprises an amino acid sequence, which differs from that of SEQ ID NO:2 in that it comprises the substitution R27K in combination with at least one additional substitution that is different from E43K, or the substitution R35K in combination with at least one additional substitution provided that the polypeptide has an amino acid sequence which is different from the amino acid sequence of wild-type human interferon β modified with the following substitutions: G7E+S12N+C17Y+R35K. The disclaimed polypeptide is disclosed by Stewart et al., DNA, Vol. 6, No. 2, 1987, pp. 119-128 ~~al, DNA Vol no2 1987 p 119-128~~ as having a retained antiproliferative activity on Daudi cells relative to their antiviral activity, but reduced overall activity as compared to wild type interferon β. The disclaimed polypeptide was not prepared with the purpose of reducing the immunogenicity and/or increasing the functional in vivo half-life and/or serum half-life, but was made in the course of a study of the structural functional relationship of interferon β.

Please replace the paragraph beginning at page 64, line 19 with the following amended paragraph:

The vector is preferably an expression vector, in which the nucleotide sequence encoding the polypeptide of the invention is operably linked to additional segments required for transcription of the nucleotide sequence. The vector is typically derived from plasmid or viral DNA. A number of suitable expression vectors for expression in the host cells mentioned herein are commercially available or described in the literature. Useful expression vectors for eukaryotic hosts, include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus. Specific vectors are, e.g., pCDNA3.1(+)\Hyg (Invitrogen, Carlsbad, CA, USA) and pCI-neo (Stratagene, La Jolla ~~Jola~~, CA, USA). Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *E. coli*, including pBR322, pET3a and pET12a (both from Novagen Inc., WI, USA), wider host range plasmids, such as RP4, phage DNAs, e.g., the numerous derivatives of

phage lambda, e.g., NM989, and other DNA phages, such as M13 and filamentous single stranded DNA phages. Useful expression vectors for yeast cells include the 2μ plasmid and derivatives thereof, the POT1 vector (US 4,931,373), the pJSO37 vector described in (Okkels, Ann. New York Acad. Sci. 782, 202-207, 1996) and pPICZ A, B or C (Invitrogen). Useful vectors for insect cells include pVL941, pBG311 (Cate et al., "Isolation of the Bovine and Human Genes for Mullerian Inhibiting Substance And Expression of the Human Gene In Animal Cells", Cell, 45, pp. 685-98 (1986), pBluebac 4.5 and pMelbac (both available from Invitrogen).

Please replace the paragraph beginning at page 68, line 30 with the following amended paragraph:

Examples of suitable yeast host cells include strains of Saccharomyces, e.g. S. cerevisiae, Schizosaccharomyces, Klyveromyces, Pichia, such as P. pastoris or P. methanolica, Hansenula, such as H. Polymorpha or Yarrowia. Methods for transforming yeast cells with heterologous DNA and producing heterologous polypeptides therefrom are disclosed by Clontech Laboratories, Inc, Palo Alto, CA, USA (in the product protocol for the Yeastmaker™ Yeast Transformation ~~Tranformation~~ System Kit), and by Reeves et al., FEMS Microbiology Letters 99 (1992) 193-198, Manivasakam and Schiestl, Nucleic Acids Research, 1993, Vol. 21, No. 18, pp. 4414-4415 and Ganeva et al., FEMS Microbiology Letters 121 (1994) 159-164.

Please replace the paragraph beginning at page 69, line 19 with the following amended paragraph:

Methods for introducing exogenous ~~exogeneous~~ DNA into mammalian host cells include calcium phosphate-mediated transfection, electroporation, DEAE-dextran mediated transfection, liposome-mediated transfection, viral vectors and the transfection methods described by Life Technologies Ltd, Paisley, UK using Lipofectamin 2000 and Roche Diagnostics Corporation, Indianapolis, USA using FuGENE 6. These methods are well known in the art and e.g. described by Ausbel et al. (eds.), 1996, Current Protocols in Molecular Biology, John Wiley & Sons, New York, USA. The cultivation of mammalian cells are conducted according to established methods, e.g. as disclosed in (Animal Cell Biotechnology, Methods and Protocols, Edited by Nigel

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Jenkins, 1999, Human Press Inc, Totowa, New Jersey, USA and Harrison MA and Rae IF, General Techniques of Cell Culture, Cambridge University Press 1997).

Please replace the header at page 72, line 4 with the following amended header:

Pharmaceutical composition ~~compositition~~ and uses of a conjugate of the invention

Please replace the paragraph beginning at page 72, line 5 with the following amended paragraph:

The IFNB molecule or conjugate of the invention is administered at a dose approximately paralleling that employed in therapy with human IFNB such as Avonex® interferon beta-1a, Rebif® interferon beta-1a and Betaseron® interferon beta-1b, or a higher dose. The exact dose to be administered depends on the circumstances. Normally, the dose should be capable of preventing or lessening the severity or spread of the condition or indication being treated. It will be apparent to those of skill in the art that an effective amount of an IFNB molecule or conjugate depends, inter alia, upon the disease, the dose, the administration schedule, whether the molecule or conjugate is administered alone or in conjunction with other therapeutic agents, the serum half-life of the compositions, and the general health of the patient.

Please replace the paragraph beginning at page 80, line 7 with the following amended paragraph:

Accordingly, this invention provides compositions and methods for treating most types of viral infections, cancers or tumors or tumour angiogenesis, Chrohn's disease, ulcerative colitis, Guillain-Barré syndrome, glioma, idiopathic pulmonary fibrosis, abnormal cell growth, or for immunomodulation in any suitable animal, preferably mammal, and in particular human. For example, the molecule or composition of the invention or conjugate of the invention may be used in the treatment of osteosarcoma, basal cell carcinoma, ovarian carcinoma, cervical dysplasia, cervical carcinoma, laryngeal papillomatosis, mycosis fungoides, glioma, acute myeloid leukemia, multiple myeloma, Hodgkin`s disease, melanoma, breast carcinoma, non-small cell lung cancer, malignant melanoma (adjuvant, late stage, as well as prophylactic), carcinoid tumour, B-cell lymphoma, T-cell lymphoma, follicular lymphoma, Kaposi's sarcoma,

chronic myelogenous leukaemia, renal cell carcinoma, recurrent superficial ~~superfiecial~~ bladder cancer, colorectal carcinoma, hairy cell leukaemia, and viral infections such as papilloma virus, viral hepatitis, herpes genitalis, herpes zoster, herpetic keratitis, herpes simplex, viral encephalitis, cytomegalovirus pneumonia, rhinovirus chronic persistent hepatitis, chronic active HCV (type I), chronic active HCV (type II) and chronic hepatitis B.

Please replace the paragraph beginning at page 81, line 5 with the following amended paragraph:

In a further aspect the invention relates to a method of treating a mammal having circulating antibodies against IFNB 1a, such as Avonex™ or Rebif®, or 1b, such as Betaseron®, which method comprises administering a variant or conjugate of the invention which has a reduced or no reaction with said antibodies. The compound is administered in an effective amount. The mammal is preferably a human being. The mammals to be treated may suffer from any of the diseases listed above for which interferon β is a useful treatment. In particular, this aspect of the invention is of interest for the treatment of multiple sclerosis (any of the types listed above), hepatitis or cancer. Furthermore, the invention relates to a method of making a pharmaceutical product for use in treatment of mammals having circulating antibodies against interferon β 1a, such as Avonex® interferon beta-1a ~~Avonex™~~ or Rebif® interferon beta-1a, or 1b, such as Betaseron® interferon beta-1b, wherein a variant or conjugate of the present invention which has reduced reaction or no reaction with such circulation antibodies (e.g. the reaction is reduced by at least 25%, such as by at least 50%, and preferably by at least 75% such as about 100% (i.e. no reaction) is formulated into an injectable or otherwise suitable formulation as further described above. The term "circulating antibodies" is intended to indicate antibodies, in particular neutralizing antibodies, formed in a mammal in response to having been treated with any of the commercially available IFNB preparations (Rebif® interferon beta-1a, Betaseron® interferon beta-1b, Avonex® interferon beta-1a).

Please replace the paragraph beginning at page 84, line 22 with the following amended paragraph:

The β-R1 gene is activated by IFNB but not by other interferons. The transcription ~~transciption~~ of β-R1 thus serves as a second marker of IFNB activation and is used to ensure that muteins retain IFNB activity. A 300 bp promoter fragment of β-R1 shown to drive interferon sensitive transcription (Rani, M.R., ~~Rani. M.R.~~ et al (1996) *JBC* 271 22878-22884) was isolated by PCR from human genomic DNA and inserted into the pGL3 basic vector (Promega). The resulting β-R1:luciferase gene is used in assays similar to the primary assay described above. In astrocytoma cells, the resulting β-R1:luciferase gene has been described to show 250-fold ~~250 fold~~ higher sensitivity to IFNB than to interferon α (Rani et al., *op. cit.* ~~al. *op cit*~~).

Please replace the paragraph beginning at page 85, line 12 with the following amended paragraph:

The reaction is visualised by addition of 0.1 mL Tetramethylbenzidine (TMB) substrate chromogen. The plates are incubated for 15 minutes in the dark at RT and the reaction is stopped by addition of stop solution. The absorbance ~~absorbanse~~ is read at 450 nm ~~450nm~~ using an ELISA reader.

Please replace the paragraph beginning at page 85, line 18 with the following amended paragraph:

The receptor binding capability of a polypeptide or conjugate of the invention can be determined using the assay described in WO 95/25170 entitled "Analysis Of IFN-β(Phe_{101}) For Receptor Binding" (which ~~Binding"(which~~ is based on Daudi or A549 cells). Soluble domains of IFNAR1 and IFNAR2 can be obtained essentially as described by Arduini et al., ~~al,~~ Protein Science, 1999, Vol. ~~vol.~~ 8, pp. 1867-1877 or as described in Example 10 ~~19~~ herein.

Please replace the paragraph beginning at page 86, line 8 with the following amended paragraph:

Reduced immunogenicity of a conjugate or polypeptide of the invention is determined by use of an ELISA method measuring the immunoreactivity of the conjugate or polypeptide relative to a reference molecule or preparation. The reference molecule or preparation is

normally a recombinant human IFNB preparation such as Avonex® interferon beta-1a, Rebif® interferon beta-1a or Betaseron® interferon beta-1b, or another recombinant human IFNB preparation produced by a method equivalent to the way these products are made. The ELISA method is based on antibodies from patients treated with one of these recombinant IFNB preparations. The immunogenicity is considered to be reduced when the conjugate or polypeptide of the invention has a statistically significant lower response in the assay than the reference molecule or preparation.

Please replace the paragraph beginning at page 88, line 11 with the following amended paragraph:

Measurement of biological half-life can be carried out in a number of ways described in the literature. One method is described by Munafo et al. ~~al~~ (European Journal of Neurology 1998, Vol. 5, No. 2, pp. ~~vol 5 No2 p~~ 187-193), who used an ELISA method to detect serum levels of IFNB after subcutaneous and intramuscular administration of IFNB.

Please replace the paragraph beginning at page 88, line 16 with the following amended paragraph:

The rapid decrease of IFNB serum concentrations after i.v. administration has made it important to evaluate biological responses to IFNB treatment. However it is contemplated that the conjugates of the present invention will have prolonged serum half lives ~~lifes~~ also after i.v. administration making it possible to measure by e.g. an ELISA method or by the primary screening assay.

Please replace the paragraph beginning at page 88, line 25 with the following amended paragraph:

Assays to assess the biological effects of IFNB such as antiviral, antiproliferative and immunomodulatory effects (as described in e.g. Annals of Neurology, Vol. 37, No. 1, pp. 7-15 ~~Neurology 1995 vol 37 No 1 p 7-15~~) can be used together with the primary and secondary screening assays described herein to evaluate the biological efficacy of the conjugate in comparison to wild type IFNB.

Please replace the paragraph beginning at page 89, line 10 with the following amended paragraph:

Transferring culture broth to one or more wells in a microtiter plate capable of immobilising the tagged polypeptide. When the tag is His-His-His-His-His-His (Casey et al., ~~al,~~ J. Immunol. Meth. ~~Meth.,~~ 179, 105 (1995)), a Ni-NTA HisSorb microtiter plate commercially available from QiaGen can be used.

Please replace the paragraph beginning at page 90, line 5 with the following amended paragraph:

The soluble domains of IFNAR1 and IFNAR2 are obtained essentially as described in Arduini et al., ~~al,~~ Protein Science (1999), Vol. ~~vol~~ 8: 1867-1877 or as described in Example 10 ~~9~~.

Please replace the paragraph beginning at page 90, line 13 with the following amended paragraph:

M-SPA-5000 from Shearwater Polymers, Inc is added at 3 different concentration levels corresponding to 5, 20 or 100 molar excess of interferon β polypeptide. The reaction time is 30 min at RT. After the 30 min reaction period, the pH of the reaction mixture is adjusted to pH 2.0 and the reaction mixture is applied to a Vydac C18 column and eluted with an acetonitrile gradient essentially as described (Utsumi et al., ~~etal,~~ J. Biochem., Vol. ~~vol~~ 101, pp. 1199-1208, (1987). Alternatively and more elegantly, an isopropanol gradient can be used.

Please replace the paragraph beginning at page 90, line 31 with the following amended paragraph:

The computer program Access (B. Lee and F. M. Richards, J. Mol. Biol. ~~F.M.Richards, J. Mol.Biol.~~ 55: 379-400 (1971)) version 2 (Copyright (c) 1983 Yale University) is ~~are~~ used to compute the accessible surface area (ASA) of the individual atoms in the structure. This method typically uses a probe-size of 1.4Å and defines the Accessible Surface Area (ASA) as the area formed by the centre of the probe. Prior to this calculation all water molecules and all hydrogen atoms are removed from the coordinate set, as are other atoms not directly related to the protein. Alternative programs are available for computing ASA, e.g. the program WhatIf G.Vriend, J.

Mol. Graph. (1990) 8, 52-56, electronically available at the WWW website at swift.embl-heidelberg.de/servers2/ (R. Rodriguez et al., CABIOS (1998) 14:523-528) ~~interface on http://swift.embl-heidelberg.de/servers2/ (R.Rodriguez *et.al.* CABIOS (1998) 14, 523-528.)~~ using the option *Accessibility* to calculate the accessible molecular surface.

Please replace the paragraph beginning at page 92, line 3 with the following amended paragraph:

The three-dimensional crystal structure of human IFNB at 2.2 Å resolution (Karpusas *et al.* Proc. Nat. Acad. Sci. USA (1997) 94:11813-11818 is available from the Protein Data Bank (PDB) (Bernstein *et.al.* J. Mol. Biol. (1977) 112 pp. 535) and electronically available via The Research Collaboratory for Structural Bioinformatics PDB at the www website at pdb.org ~~http://www.pdb.org/~~ under accession code 1AU1. This crystal structure contain two independent molecules of human IFNB; ~~IFNB~~ in this example the A molecule is used.

Please replace the paragraph beginning at page 100, line 5 with the following amended paragraph:

The cell line CHO K1 [p22]-E4 (ATCC # CCL-61) stably expressing human interferon β was passed 1:10 from a confluent culture and propagated as adherent cells in T-25 flasks in serum containing medium (MEMα w/ ribonucleotides and deoxyribonucleotides (Gibco/BRL Cat # 32571), 10% FCS (Gibco/BRL Cat # 10091), penicillin and streptomycin (Gibco/BRL Cat # 15140-114) until confluence. The media was then changed to serum free media (RenCyte CHO; MediCult Cat.# 22600140) for 24 hours before including 5 mM Sodium Butyrate (Merck Cat # 8.17500) during a medium change. The cells were then allowed to express interferon β for 48 hours prior to harvest of the medium. The interferon β concentration in the duplicate cultures was ~~were~~ determined to be 854,797 IU/ml (with lower and upper 95% confidence interval at 711,134 IU/ml and 1,032,012 IU/ml) respectively).

Please replace the paragraph beginning at page 108, line 10 with the following amended paragraph:

In the presence of 200 ng/mL polyclonal rabbit anti-serum the activity of the wild type interferon β protein was reduced 11.8 times whereas the activity of the glycosylated interferon β variant only was reduced 3.0 times. Thus the degree of antibody recognition of the interferon β variant was reduced by 75% of the wt level, see Table 1 below. These results demonstrate that the recognition of the glycosylated mutant interferon β by polyclonal antibodies raised in animals immunised with wild-type human interferon β is highly reduced. Thus, a large portion of the immunogenic epitopes in wild-type human interferon β has ~~have~~ been removed/shielded by the modifications made in the variant molecule.

Please replace the paragraph beginning at page 113, line 17 with the following amended paragraph:

Hydroxyapatite chromatography is an efficient means for separation of IFNB glycoforms and e.g. obtaining ~~obtain~~ glycoforms with fully utilized glycosylation sites. This is illustrated in the present example.

Please replace the paragraph beginning at page 115, line 15 with the following amended paragraph:

CHOK1 cells were transfected with plasmids encoding two hyper-glycosylated IFNB variants: [S2N, N4T, Q51N, E53T]IFNB (PF276) and [S2N, N4T, C17S, Q51N, E53T]IFNB (PF279). Confluent stable primary transfectionpools were expanded into four T-175 flasks each. At confluency, the flasks were shifted from serum containing medium to a serum-free medium based on DMEM/F-12 medium (Lifetechnologies ~~Lifetecnologies~~ #21045-025) supplemented with 1/100 ITSA (Life Technologies #51300-044) and 1/1000 Ex-Cyte (Serologicals Corp. #81-129). Every day, in 15 days, 120 ml of each variant was harvested and frozen at –80°C.

Please replace the paragraph beginning at page 117, line 23 with the following amended paragraph:

A protein solution of 0.1 mg/ml in 20 mM sodium phosphate, pH 7.0 was PEGylated with SCM-PEG, 20K, with 0.75 times molar surplus of PEG to possible PEGylation sites, i.e.

lysines and N-terminus. After incubation for 30 min at room temperature ~~termperature~~, the reaction was quenched by addition of a surplus of 20 mM glycine, pH 8.0. The reaction mixture contained a mixture of mono-, di- and un-pegylated material. Mono-pegylated material was separated from other species using either cation exchange chromatography or size-exclusion chromatography or a combination of both. pH in the PEGylation solution was adjusted to pH 2.7 and the sample was applied on a SP-Sepharose HR (Pharmacia) column equilibrated with 20 mM sodium citrate, pH 2.7. The pegylated protein was eluted from the column with 50 mM sodium acetate containing 1 M sodium chloride and applied on a size-exclusion column, Sephacryl S-100, ((16/60) Pharmacia) equilibrated with 100 mM sodium acetate, 200 mM sodium chloride, pH 5.5. Fractions containing mono-pegylated material were pooled and characterized further.

Please replace the paragraph beginning at page 119, line 26 with the following amended paragraph:

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application ~~applica-tion~~ and scope of the appended claims. For example, all the techniques and apparatus described above may be used in various combinations. *All publications, patents, patent applications, and/or other documents cited in this application are incorporated herein by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, and/or other document were individually indicated to be incorporated herein by reference in its entirety for all purposes.*